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TURBIDIMETRIC METHOD FOR THE DETERMINATION OF BETA-LIPOPROTEIN
AND CHYLOMICRONS IN BLOOD SERUM AND TISSUES

[Following is the translation of an article by
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Medicine, AMU USSR, Leningrad, published in the
Russian-language periodical Laboratornoye Delo (Labor-
atory Affairs), No 5, 1966, pages 276--280. It was
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In 1955 Brunstein and Samaille [1] proposed a rapid and simple
turbidimetric method for the determination of beta-lipoprotein in
blood serum. It was based on the selective precipitation of the
latter with the help of dextran sulfate and heparin in the presence
of ions of Ca, Mn, Co, and Ni. If the appropriate conditions are
observed insoluble complex compounds of beta-lipoproteins with
dextran sulfate and heparin do not fall out immediately into the
precipitate, but cause a uniform turbidity of the solution. The
degree of turbidity corresponds to the concentration of beta-
lipoproteins.

The proposed turbidimetric method for the determination of
beta-lipoprotein turned out to be suitable for the investigation
of the blood of man and the majority of animals [2]. However,
the following difficulties were encountered in the quantitative
evaluation of data obtained by this method. First of all it turned
out to be impossible to construct a standard curve based on dry
beta-lipoprotein, as this is suggested by M. Ledvina [3] for ex-
ample. Until now, no one has been able to obtain pure dry and
non-denatured beta-lipoprotein. The fact is that beta-lipoproteins
are very unstable compounds and are denatured during drying and
freezing. Secondly, dextran sulfate, heparin, and other sulfated
polyanions precipitate lipoproteins with a density of 1.063 and
lower [4,5], i.e., not only beta-lipoproteins, but also chylomicrons.
Therefore, according to our observations an analysis of blood serum
by the turbidimetric method, especially lipemic, without the removal
of chylomicrons leads to overstated results.

In the present work a method is proposed for the separate
determination of beta-lipoproteins and chylomicrons, and also a
method is cited for the construction of a standard curve which would
make it possible to obtain more accurate data on the content of beta-
lipoproteins.

For total determination of beta-lipoproteins and chylomicrons to 2 ml of an 0.025 M solution of CaCl_2 we add 0.2 ml of the serum to be tested and determine the optical density (D_1) in a PEK M-1 photoelectric colorimeter in a cuvette with a thickness of 0.5 cm with a red light filter (700 nm). Then to the cuvette we add 0.04 ml of a 1% solution of heparin * and the mixture is blended. Here the beta-lipoproteins and chylomicrons transform into an insoluble condition in the form of a complex with heparin, causing a uniform turbidity of the solution and thus increasing optical density. The latter is determined 4 minutes (exactly!) after addition of the heparin (D_2). The difference $D_2 - D_1$ belongs to the optical density caused by lipo-proteins and chylomicrons. In the case of highly lipemic serum distilled water is used to dilute the latter.

* 1 mg of heparin is equal to 130 ME [mass unit].

Since heparin added to blood serum causes the precipitation not only of beta-lipoproteins, but also of chylomicrons, in order to obtain data about the content of beta-lipoproteins in serum, especially lipemic, it is necessary first of all to separate the chylomicrons from the beta-lipoproteins. For this purpose to 5 ml of serum we add 1.4 ml of a solution of NaCl with a specific gravity of 1.006 (0.85% solution). The solution of NaCl was added very slowly and carefully from a hyperdermic needle over the wall of the test tube so that it stratified on the serum. Then it was centrifuged at 10,000 g for 10 minutes. The chylomicrons rose to the surface in the form of a thin film or ring, if they were few, and the lipoproteins remained in the serum. The solution and serum were not mixed. With a hyperdermic the serum was carefully drawn out from under the saline solution and in it a determination was made of the content of beta-lipoproteins by the above-stated method.

The content of chylomicrons was established by the difference between the total content of beta-lipoproteins and chylomicrons on the one hand and the content of beta-lipoproteins on the other.

Quantitative evaluation of beta-lipoproteins is made by a standard curve which is constructed in the following manner. Serum with a high content of beta-lipoproteins (it is desirable that optical density, determined by the turbidimetric method, comprise approximately 0.7—0.8), after it has been freed of chylomicrons, is diluted with water by 2, 3, and 4 times. From each diluted sample of serum 0.2 ml is taken for the determination of beta-lipoproteins by the turbidimetric method, and 10 ml of initial and diluted sera are used for the precipitation of beta-lipoproteins.

For this, to the stated volume of serum, which was placed in a centrifuge test tube, 100 ml of distilled water, 2.5 ml of a 2 M solution of CaCl_2 , and 0.8 ml of a commercial solution of heparin (100-100 units in 5 ml) are added. The mixture is stirred thoroughly and placed in a refrigerator at $2-5^\circ$ for an hour. Then the samples are centrifuged in a refrigerated centrifuge for 45 minutes at 3000 rpm. As a result a thick precipitate of beta-lipoproteins is formed. The supernatant liquid is decanted and the beta-lipoproteins are dissolved in 2 ml of a 5% solution of NaCl and transferred quantitatively into special beakers for dialysis. These are made from flexiglas and have a screw-on ring on the bottom for fixation of a cellophane membrane.

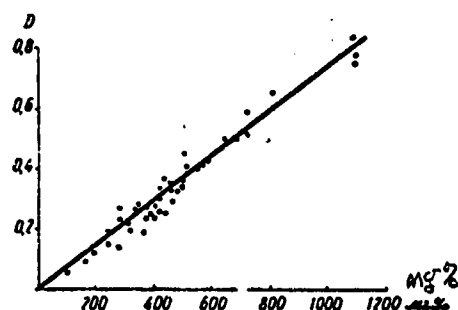


Figure 1. Standard curve for determination of the content of beta-lipoproteins and chylomicrons. Explanation in the text.

The centrifuge test tubes were rinsed twice with 0.5 ml of distilled water, which was decanted into the same beakers for dialysis. Dialysis was conducted at $2-5^\circ$ for 48 hours against distilled water, changing the latter 2-3 times. Then the contents of the beakers were transferred into weighing bottles and dried in a dessicator at $70-80^\circ$ until a uniform weight was reached. Having determined the weight of beta-lipoproteins in each sample, we cancel out the curve of dependence of the previously determined optical density on the weight of beta-lipoproteins. Figure 1 shows the standard curve for the determination of beta-lipoproteins in the blood serum of rabbits. The proposed curve holds true for beta-lipoproteins from the serum of both man and animals (rabbits, dogs, and others).

Tissues do not contain chylomicrons and therefore all lipoproteins with a density of 1.063 and lower represent compounds which are close or analogous to the beta-lipoproteins of blood.

For determination of beta-lipoproteins a portion of tissue was crushed with quartz sand in the cold in a preliminarily cooled porcelain mortar. Then a veronal-medinal buffer (pH 8.6, ionic strength 0.1) was added there in a 5-10-fold amount. The tissue was

thoroughly crushed in the buffer solution to a homogeneous mass, which was transferred into centrifuge test tubes and centrifuged at 3000 rpm for 10—15 minutes. The extract was poured off and the beta-lipoproteins in it determined. For this purpose to 2 ml of an 0.025 M solution of CaCl_2 we added 0.2 ml of resulting extract and 0.04 ml of a 1% solution of heparin, the mixture was thoroughly mixed, and then the optical density (D_2) was established in a PEK-M-1 photoelectric colorimeter in a cuvette with a thickness of 0.5 cm with a red light filter (700 nm), just as in the determination of beta-lipoproteins in serum. Then the entire mixture was transferred from the cuvette into a test tube and again centrifuged at 3000 rpm for 15—20 minutes. All the beta-lipoproteins precipitated. The supernatant liquid was poured off again into a cuvette and the optical density (D_1) was determined. The difference $D_2 - D_1$ is attributed to the optical density caused by the beta-lipoproteins of tissue.

Table 1

Content of beta-lipoproteins in the blood serum of man and animals (in mg%)

Человек	Кролик	Морская свинка	Суслик	Крыс	Мышь	Собака
545	145	165	530	105	215	185
560	170	115	560	110	355	240
615	170	165	580	125	390	290
660	220	220	615	125	515	435
660	220	165	660	125	565	575
815	305	360	690	130	—	—
870	330	265	800	165	—	—
905	360	330	900	165	—	—
925	500	260	1110	—	—	—
945	590	—	1200	—	—	—
1020	—	—	1410	—	—	—

Key: (a) Man; (b) Rabbit; (c) Guinea pig; (d) Suslik; (e) Rat; (f) Pig; (g) Dog.

A quantitative evaluation was made based on the curve obtained for the beta-lipoproteins of serum. We determined the beta-lipoproteins in the aorta and liver of rabbits. In the first case the dilution of tissue comprised 1:4, and in the second - 1:9. If there was much beta-lipoprotein in the extract being investigated it was necessary to dilute it several times.

Table 1 presents the content of beta-lipoproteins in the blood serum of man and some animals according to data from the proposed method.

Table 2

Content of beta-lipoproteins and chylomicrons in the blood serum of rabbits with experimental atherosclerosis (in mg%)

β-Lipoproteins plus chylomicrons (a)	β-Lipoproteins (b)	Chylomicrons (c)
1000	700	300
1150	800	350
1350	1300	50
1375	1150	225
1975	1800	175
2000	1775	225
2100	1950	150
2100	1600	500
2325	2200	125
2150	1950	500
3025	2075	950
3125	2150	975
3450	3125	325

Key: (a) Beta-lipoproteins plus chylomicrons; (b) Beta-lipoproteins; (c) Chylomicrons.

Data from Table 1 are in good agreement with the generally accepted indices of the content of beta-lipoproteins in blood which are obtained with the help of preparing ultracentrifuging and other methods [6].

As a rule in blood taken from man or animals on an empty stomach the content of chylomicrons was exceedingly low or they were absent. However, after the taking of fatty food or the development of experimental atherosclerosis as a result of feeding the animals cholesterol, the level of chylomicrons can increase significantly.

Table 2 presents data on the content of beta-lipoproteins and chylomicrons in the blood serum of rabbits with experimental atherosclerosis.

As can be seen from Table 2, during experimental atherosclerosis the content of chylomicrons and especially of beta-lipoproteins is increased, while the latter is always greater than chylomicrons. No specific dependence is noted between the content of beta-lipoproteins and chylomicrons. The data cited in Table 2 also testify that in a number of cases a significant error can develop if the beta-lipoproteins were determined with a consideration of the content of chylomicrons.

Table 3 presents data on the content of beta-lipoproteins in the aorta of healthy rabbits.

Table 3

Content of beta-lipoproteins in the aorta of rabbits (in mg%)

β-Lipoproteins (a)	β-Lipoproteins (b)
1	298
2	300
3	357
4	420
5	500
6	500
7	630
8	711
9	797

Key: (a) No. of animal; (b) Beta-lipoproteins.

It can be seen from table 3 that the content of beta-lipoproteins in the aorta of rabbits exceeds their level in the blood (see Table 1).

Still more beta-lipoproteins are found in the liver, where they are formed. Based on data obtained by our method the content of beta-lipoproteins in the liver of a rabbit comprises 1500—2000 mg.

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